PATENT
0459-0490P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	Lorenzo Williams	Conf.:	8775
Appl. No.:	09/680,471	Group:	1743
Filed:	October 6, 2000	Examiner:	Y.G. GAKH

For: A METHOD FOR SYNTHESIS, SEPARATION AND SCREENING OF A PLURALITY OF COMPOUNDS IN THE SAME BULK OF A STATIONARY PHASE

Declaration of Lorenzo Williams under 37 C.F.R. §1.131

1. I, Lorenzo Williams of SINTEF Applied Chemistry located at Postboks 124 Blindem, N-0314 Oslo, Norway declare as follows:

2. I am the inventor of the subject matter of U.S. patent application serial number 09/680,471 (the '471 application), which was filed October 6, 2000. As an inventor, I am very familiar with the subject matter of the invention and the prosecution history of this application.

3. I was working on the subject matter of the invention disclosed in the '471 application at least as early as April 24, 1998 in Norway. (Please see Exhibit A attached

hereto, page one, the cover page of the laboratory notebook containing the recordings of my work on the invention.)

4. The invention currently claimed in the above referenced application is directed to a method for preparing and screening a plurality of compounds, said method performed in or on a bulk of a stationary phase, the method comprises the sequential steps of: (a) performing a synthesis of compounds by a chemical reaction performed in the bulk of a stationary phase, (b) separating the compounds in or on the same bulk of the stationary phase using a mobile phase; and
(c) screening of the separated compounds in or on the same bulk of stationary phase, wherein said screening involves biological or biochemical methods; wherein the stationary phase is a thin-layer chromatography plate and wherein the stationary phase is suitable for sequential synthesis (claim 1).

5. I invented steps (a) and (b) of the claimed process at least as early as May 12, 1998 as evidenced by notebook pages 4-7 of the Exhibit A. The pages 4 and 5 relate to an experiment where 4 amines are spotted neat onto a TLC plate. Benzyl bromide is then spotted on top of the amines and a reaction begins. Elution of the plate with 10% MeOH-CH_2Cl_2 and visualization under UV (254 nm) and also with

iodine and ninhydrin stains produces the plate as seen in the picture diagram on page 4 of the laboratory notebook in Exhibit A.

6. 12 lanes can be seen on the plate representing the following from left to right:
Pyrrolidine (reference), reaction of pyrrolidine with benzyl bromide, benzyl bromide (reference)
Morpholine (reference), reaction of morpholine with benzyl bromide, benzyl bromide (reference)
n-Butylamine (reference), reaction of n-butylamine with benzyl bromide, benzyl bromide (reference)
Benzylamine (reference), reaction of benzylamine with benzyl bromide, benzyl bromide (reference)

7. The reaction occurred in each case as evidenced by a new spot. Note that the starting amines are all baseline in this eluent and that benzyl bromide can be seen at the top of the plate. Spots in between the baseline and benzyl bromide are reaction products from the 4 reactions.

8. Pages 6 and 7 of the laboratory notebook, Exhibit A, relate to an experiment monitoring the reaction time for 2 of the reactions, the reaction of pyrroline and morpholine with

benzyl bromide. The reaction does not progress further after 2 minutes. The product spots are in the middle of the plate.

9. Confirmation of the structure of these products was made at a later date. The confirmation of the structures corresponds to step (c) of the claimed process.

I declare that all statements made herein are of my own knowledge and are true to the best of my belief. I am aware that willful false statements or representations are punishable by fine, imprisonment or both pursuant to 18 U.S.C. section 1001 and may jeopardize the validity of any patents issued.

Date: 25th June, 2004

Lorenzo Williams

Lorenzo Williams, Ph.D.

SINTEF
Kjemi

Nr.:

LABORATORIEJOURNAL
for

Navn: Lorenzo Williams

Gruppe: 6650

Utlevert: 24/4-98

Avsluttet:

Prosjekt:
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Kombinatorisk syntese
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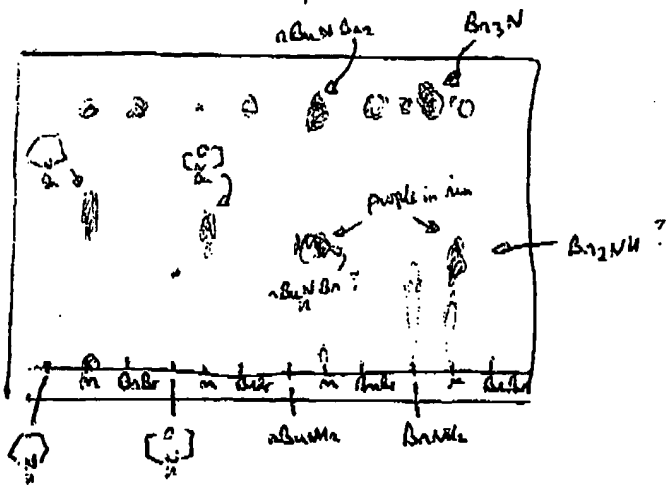
MeOH-CH_2Cl_2 ~~95~~

10 % ~~EtOAc/Hex~~ (UV, I_2, Ninh)



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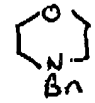
Amines → /			$nBuNH_2$	$BnNH_2$
$BnBr$			$nBuNHBn$	$BnNHBn$
			$nBuNBn_2$	Bn_2NH

Procedure

Ca. 5 µl of neat n amine was spotted onto ca. 5µl of neat $BnBr$ on a tlc plate and the plate eluted. Tlc plate ca 5cm x 7cm kieselgel 60 F_{254} (105.031-0 from Merck)

From the tlc appeared as though all 6 products were obtained.

Lorentz Tottrup 25/4/98

6 $\rho = \frac{m}{v}$

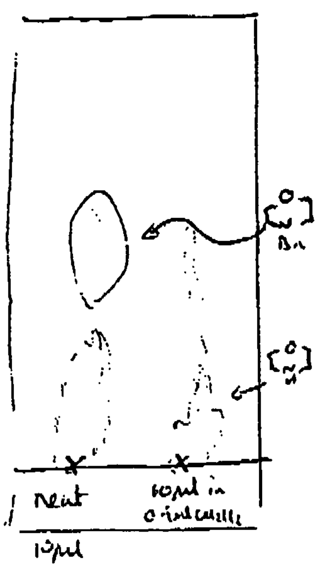
pyrrolidine bp 87-88°C ; Mr 71.12 ; ρ 0.852 11.98×10⁻³
BnBr bp 198-199°C ; Mr 171.04 ; ρ 1.438 8.4×10⁻³
morpholine bp 129°C ; Mr 87.12 ; ρ 0.999 11.47×10⁻³

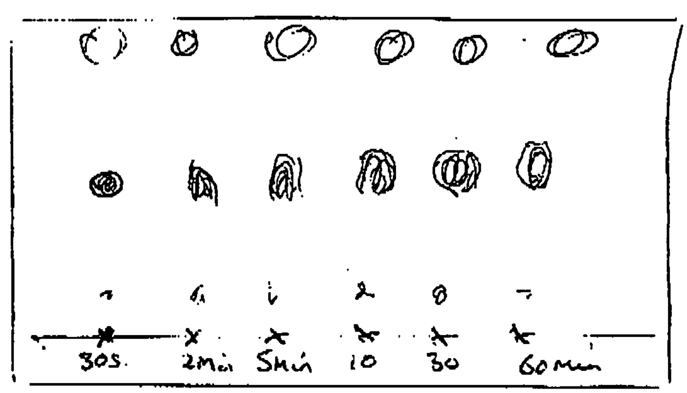
μl morpholine by volume	= 0.2μl BnBr by volume (eq/ml)
m.eq. 1.36	1
2.72	1
4.08	1
1.36	2
1.36	4

neat spotting v dilution 10μl in ca. 0.1 ml CH_2Cl_2 (as morpholine 1.36:1)
time before elution 30s, 2min, 5min, 10min, 30min, 60min (as morpholine 1.36:1)

NB 10μl appears to correspond to 6mg morpholine + 4mg BnBr in 3:1 EtOAc-hex (10). (UV - also I₂, much)

5% MeOH - CH_2Cl_2





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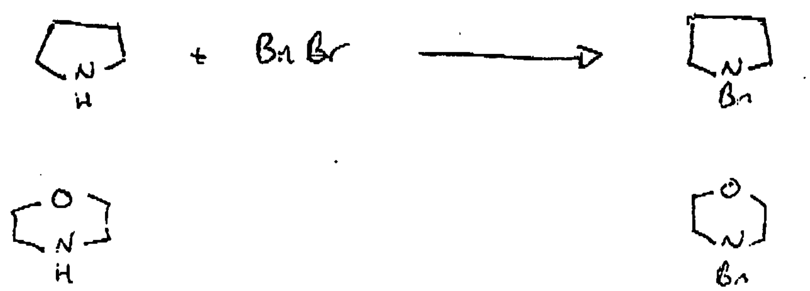

Morpholine is more appropriate to optimise reaction because of higher b.p.
10µl morpholine + 10µl BnBr clearly overloads a plate with 0.25mm thickness. Reaction appears to be far more effective with neat loading - note xs of amine here, all BnBr consumed.

Lorenzo [illegible] 12/5/98